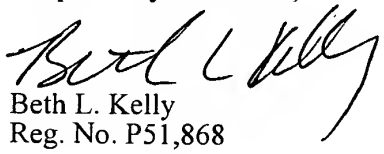
Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-33, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the Specification and Claims by the current Amendment. The attached pages are captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**" As a convenience to the Examiner, a complete set of the Claims, as amended herein, is also attached to this Amendment as an Appendix entitled "**PENDING CLAIMS WITH ENTRY OF THE AMENDMENT**."

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Paragraph beginning at line 9 of page 3 has been amended as follows:

In one aspect, the present invention provides for oligonucleotide primers that comprise in the following order from 5' to 3': a phage-encoded RNA polymerase recognition sequence, a spacer sequence comprising a sequence of from 12 to 20 nucleotides that consists of one nucleotide type or two different nucleotide types, and a target complementary sequence which can bind a segment of a target nucleic acid. In certain embodiments, the spacer ~~space~~ sequence comprises a nucleotide sequence having the formula $(XY)_n$, wherein n is from 6 to 10, wherein X and Y are independently selected from the group consisting of an adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide, wherein X and Y are not the same (SEQ ID NO:9). In certain preferred embodiments, X is an adenine nucleotide and Y is a guanine nucleotide (SEQ ID NO:29). In other embodiments, the spacer sequence comprises a nucleotide sequence having the formula $(X)_n$, wherein n is from 12 to 20, wherein X is selected from the group consisting of an adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide (SEQ ID NO:30).

Paragraph beginning at line 26 of page 4 has been amended as follows:

Typically, these methods further involve repetitively carrying out steps i to vii. For example, steps i to vii can be carried out one, two, etc. When the first or second spacer comprises a nucleotide sequence having the formula $(XY)_n$ (SEQ ID NO:9), as described above, then the rNTPs represented in the spacer sequences should be present in the reaction mixture. For example if a spacer sequence is $(AG)_{12\text{-}20}$ (SEQ ID NO:5) then rATP and rGTP should be present in the reaction mixture. Similarly, the first or said second spacer sequence can comprise a nucleotide sequence having the formula $(X)_{12\text{-}20}$

(SEQ ID NO:30), where X is a dNTP. Then, the corresponding rNTP should be present in the reaction mixture.

Paragraph beginning at line 27 of page 8 has been amended as follows:

Figure 2 depicts an embodiment of the first half of the amplification cycle from primer binding to the antisense transcription product through production of sense transcription product. The entry point into the amplification cycle from the intermediate duplex is through the sense transcription product. Spacer sequences = SEQ ID NOS:31 and 32.

Paragraph beginning at line 31 of page 8 has been amended as follows:

Figure 3 depicts an embodiment of the second half of the amplification cycle from primer binding to the sense transcription product through production of antisense transcription product. The amplification cycle consists of alternating between production of sense transcription products and antisense transcription products. Spacer sequences = SEQ ID NOS:31 and 32.

Paragraph beginning at line 5 of page 16 has been amended as follows:

Essentially, the spacer sequence can be composed of any arrangement of up to two different nucleotide types. Thus, the spacer sequence can be composed of just one nucleotide, e.g., adenine, thymidine, guanosine, or cytosine. Ergo, in some embodiments, the spacer sequence is a homopolymer with the formula $(X)_n$, where n is from 12 to 20, and where X is selected from the group consisting of an adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide (SEQ ID NO:30). Examples of these spacer sequence embodiments include, without limitation, $(A)_{12\text{-}20}$ (SEQ ID NO: 5), $(T)_{12\text{-}20}$ (SEQ ID NO: 6), $(C)_{12\text{-}20}$ (SEQ ID NO: 7), and $(G)_{12\text{-}20}$

(SEQ ID NO: 8). In other embodiments, the spacer sequence is a combination of two different types of nucleotides, e.g., A and T, A and G, A and C, T and G, T and C, and G and C. For example, in certain embodiments, the spacer sequence comprises a nucleotide sequence having the formula $(XY)_n$ ~~(SEQ ID NO: 9)~~, where n is preferably from 6 to 10 (SEQ ID NO:9). The nucleotides X and Y for these spacer sequence embodiments are independently selected from the group consisting of an adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide, and X and Y are not the same. In preferred embodiments, X is an adenine nucleotide and Y is a guanine nucleotide (SEQ ID NO:29).

Paragraph (Table 2.) beginning at line 20 of page 41 has been amended as follows:

Table 2.

~~Table 2~~ Spacer Variant in Amp011

Primer	Spacer Structure	Sequence	SEQ ID NO:
AMP011 ~~Amp011~~	Standard	GGGAGAGAGAGAGAGAGA	27
AMP011Sc ~~Amp011Sc~~	Modified	GGGAGAAGGAGAAAAAGA	28
	Difference	* * * *	

In the Claims:

Claims 2-6, 9-11 and 13-14 have been amended as follows:

2. (Amended) The primer of claim 1, wherein said spacer sequence comprises a nucleotide sequence having the formula $(XY)_n$,

wherein n is from 6 to 10,

wherein X and Y are independently selected from the group consisting of an adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide,

wherein X and Y are not the same, as set forth in SEQ ID NO:9.

3. (Amended) The primer of claim 2, wherein X is an adenine nucleotide and Y is a guanine nucleotide, as set forth in SEQ ID NO:29.

4. (Amended) The primer of claim 3, wherein n is 9, as set forth in SEQ ID NO:31.

5. (Amended) The primer of claim 1, wherein said spacer sequence comprises a nucleotide sequence having the formula $(X)_n$,

wherein n is from 12 to 20,

wherein X is selected from the group consisting of an adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide, as set forth in SEQ ID NO:30.

6. (Amended) The primer of claim 5, wherein n is 18, as set forth in SEQ ID NO:33.

9. (Amended) The method of claim 7, wherein said first or said second spacer sequence comprises a nucleotide sequence having the formula $(XY)_n$,

wherein n is from 6 to 10,

wherein X and Y are independently selected from the group consisting of an adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide,

wherein X and Y are not the same, as set forth in SEQ ID NO:9.

10. (Amended) The method of claim 9, wherein X is an adenine nucleotide and Y is a guanine nucleotide, as set forth in SEQ ID NO:29.

11. (Amended) The method of claim 10, wherein n is 9, as set forth in SEQ ID NO:31.

13. (Amended) The method of claim 7, wherein said first or said second spacer sequence comprises a nucleotide sequence having the formula $(X)_n$,

wherein n is from 12 to 20,

wherein X is selected from the group consisting of an adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide, as set forth in SEQ ID NO:30.

14. (Amended) The method of claim 13, wherein n is 18, as set forth in SEQ ID NO:33.

PENDING CLAIMS WITH ENTRY OF THE AMENDMENT

1. (As filed) An oligonucleotide primer comprising in the following order from 5' to 3':

a phage-encoded RNA polymerase recognition sequence,

a spacer sequence comprising a sequence of from 12 to 20 nucleotides that consists of one nucleotide type or two different nucleotide types, and

a target complementary sequence which can bind a segment of a target nucleic acid.

2. (Once Amended) The primer of claim 1, wherein said spacer sequence comprises a nucleotide sequence having the formula $(XY)_n$,

wherein n is from 6 to 10,

wherein X and Y are independently selected from the group consisting of an adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide,

wherein X and Y are not the same, as set forth in SEQ ID NO:9.

3. (Once Amended) The primer of claim 2, wherein X is an adenine nucleotide and Y is a guanine nucleotide, as set forth in SEQ ID NO:29.

4. (Once Amended) The primer of claim 3, wherein n is 9, as set forth in SEQ ID NO:31.

5. (Once Amended) The primer of claim 1, wherein said spacer sequence comprises a nucleotide sequence having the formula $(X)_n$,

wherein n is from 12 to 20,

wherein X is selected from the group consisting of an adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide, as set forth in SEQ ID NO:30.

6. (Once Amended) The primer of claim 5, wherein n is 18, as set forth in SEQ ID NO:33.

7. (As filed) A method of amplifying a target nucleic acid in an aqueous solution with a first and a second primer, said method comprising:

i.) transcribing an intermediate duplex with a phage-encoded RNA polymerase to form a sense transcription product having a 5' end and a 3' end,

wherein said intermediate duplex comprises a double-stranded molecule, wherein said double-stranded DNA molecule comprises a first and a second strand,

wherein said first strand comprises in the following order from 5' to 3:

a phage-encoded RNA polymerase recognition sequence,

a first spacer sequence comprising a sequence of from 12 to 20 nucleotides that consists of one nucleotide type or two different nucleotide types, and

a first target complementary sequence which can bind to a segment of said target nucleic acid,

wherein said second strand comprises in the following order from 5' to 3':

a second target complementary sequence which can bind to a segment of said target nucleic acid,

a second spacer sequence comprising a sequence of from 12 to 20 nucleotides that consists of one nucleotide type or two different nucleotide types, and

a phage-encoded RNA polymerase recognition sequence,

wherein said transcribing takes place in the presence of Mn^{++}, with all four dNTPs, and with those rNTPs represented in said first spacer sequence;

ii.) hybridizing said second primer to said sense transcription product to form a second primer-sense transcription product complex,

wherein said second primer comprises in the following order from 5' to 3':

a phage-encoded RNA polymerase recognition sequence,

said second spacer sequence, and

said second target complementary sequence which can bind to a 3' segment of said target nucleic acid;

iii.) extending said second primer-sense transcription product complex with a Reverse Transcriptase that lacks RNAseH activity to form a first amplification duplex;

iv.) transcribing said first amplification duplex with a phage-encoded RNA polymerase, in the presence of Mn^{++}, with all four dNTPs, and with those rNTPs represented in said second spacer sequence, to form an antisense transcription product;

v.) hybridizing said first primer to said antisense transcription product to form a first primer-antisense transcription product complex,

wherein said first primer comprises in the following order from 5' to 3':

a phage-encoded RNA polymerase recognition sequence,

said first spacer sequence, and

said first target complementary sequence which can bind to a 5' segment of said target nucleic acid;

vi.) extending said second primer-antisense transcription product complex with a Reverse Transcriptase that lacks RNAseH activity to form a second amplification duplex; and

vii.) transcribing said second amplification duplex with a phage-encoded RNA polymerase, in the presence of Mn^{++}, with all four dNTPs, and with those rNTPs represented in said first spacer sequence to form said sense transcription product.

8. (As filed) The method of claim 7, wherein the method further comprises repetitively carrying out steps i to vii.

9. (Once Amended) The method of claim 7, wherein said first or said second spacer sequence comprises a nucleotide sequence having the formula $(XY)_n$,

wherein n is from 6 to 10,

wherein X and Y are independently selected from the group consisting of an adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide,

wherein X and Y are not the same, as set forth in SEQ ID NO:9.

10. (Once Amended) The method of claim 9, wherein X is an adenine nucleotide and Y is a guanine nucleotide, as set forth in SEQ ID NO:29.

11. (Once Amended) The method of claim 10, wherein n is 9, as set forth in SEQ ID NO:31.

12. (As filed) The method of claim 10, wherein the rNTPs are rATP and rGTP.

13. (Once Amended) The method of claim 7, wherein said first or said second spacer sequence comprises a nucleotide sequence having the formula $(X)_n$,

wherein n is from 12 to 20,

wherein X is selected from the group consisting of an adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide, as set forth in SEQ ID NO:30.

14. (Once Amended) The method of claim 13, wherein n is 18, as set forth in SEQ ID NO:33.

15. (As filed) The method of claim 7, wherein said sense and antisense transcription products comprise a nucleic acid strand comprising both ribonucleotides and deoxyribonucleotides.

16. (As filed) The method of claim 7, wherein said first and said second amplification duplexes consist of deoxyribonucleotides and ribonucleotides.

17. (As filed) The method of claim 7, wherein said method is carried out at a single temperature.

18. (As filed) The method of claim 7, wherein said method is carried out at a single temperature of between 25 °C and 55 °C.

19. (As filed) The method of claim 1, wherein the method is carried out at a single temperature of greater than 50 °C.

20. (As filed) The method of claim 7, wherein said intermediate duplex comprises a double-stranded DNA comprising one complete primer sequence followed by the entire sequence that is to amplified.

21. (As filed) The method of claim 7, wherein said intermediate duplex is formed from double-stranded DNA, single-stranded DNA, or RNA.

22. (As filed) The method of claim 7, wherein said intermediate duplex is formed by the process comprising the following steps of:

denaturing a double-stranded DNA target to form an upper strand and a lower strand;

hybridizing said first primer to said lower strand to form a first primer-lower strand complex;

extending said first primer-lower strand complex with a Reverse Transcriptase that lacks RNAseH activity or with a DNA Polymerase to form a first long sense strand product-lower strand complex;

denaturing said first long sense strand product-lower strand complex into a first long sense strand product and said lower strand;

hybridizing said second primer to said first long sense strand product to form a second primer-first long sense strand product; and

extending said first primer-first long antisense strand product with a Reverse Transcriptase that lacks RNAseH activity or with a DNA Polymerase to yield said intermediate duplex.

23. (As filed) The method of claim 7, wherein said intermediate duplex is formed by the process comprising the following steps of:

denaturing a double-stranded DNA target to form an upper strand and a lower strand;

hybridizing said first primer to said lower strand to form a first primer-lower strand complex;

extending said first primer-lower strand complex with a Reverse Transcriptase that lacks RNAseH activity or with a DNA Polymerase to form a first long sense strand

product-lower strand complex, wherein said first long sense strand product has a 5' and a 3' end;

displacing said first sense strand product from said lower strand by:

hybridizing a bumper oligonucleotide to a subsequence on said lower strand adjacent to said 5' end of said first sense strand product on the first sense strand product-lower strand complex;

extending said bumper oligonucleotide with a Reverse Transcriptase that lacks RNAseH activity or with a DNA Polymerase, thereby displacing said first sense strand product;

hybridizing said second primer to said first long sense strand product to form a second primer-first long sense strand product; and

extending said first primer-first long antisense strand product with a Reverse Transcriptase that lacks RNAseH activity or with a DNA Polymerase to yield said intermediate duplex.

24. (As filed) The method of claim 7, wherein said intermediate duplex is formed by the process comprising the following steps of:

hybridizing said second primer to a target RNA molecule to form a second primer-RNA template complex;

extending said second primer-target RNA molecule complex with a Reverse Transcriptase that lacks RNAseH activity or a DNA Polymerase to form a first long antisense strand product-template complex, wherein said first long antisense strand product has a 5' and a 3' end;

displacing said first long antisense strand product from said target RNA molecule by:

hybridizing a bumper oligonucleotide to a subsequence on said target RNA molecule adjacent to said 5' end of said first sense strand product on the first sense strand product-lower strand complex;

extending said bumper oligonucleotide with a Reverse Transcriptase that lacks RNAseH activity or with a DNA Polymerase, thereby displacing said first long antisense strand product;

hybridizing said first primer to said first long antisense strand product to form a first primer-first long antisense strand product complex; and

extending said first primer-first long antisense strand product with a Reverse Transcriptase that lacks RNAseH activity or with a DNA Polymerase to yield said intermediate duplex.

25. (As filed) The method of claim 7, wherein said intermediate duplex is formed by the process comprising the following steps of:

hybridizing said second primer to a single-stranded target RNA molecule to form a second primer-RNA template complex;

extending said second primer-RNA template complex with a Reverse Transcriptase that lacks RNAseH activity or a DNA Polymerase to form a first long antisense strand product-template complex;

denaturing said first long antisense strand product-RNA template complex into a first long antisense strand product and said single-stranded RNA molecule;

hybridizing said first primer to said first long antisense strand product to form a first primer-first long antisense strand product complex; and

extending said first primer-first long antisense strand product with a Reverse Transcriptase that lacks RNAseH activity or with a DNA Polymerase to yield said intermediate duplex.

26. (As filed) The method of claim 7, wherein said phage-encoded RNA polymerase is polymerase selected from the group consisting of : a T7 RNA polymerase, a T4 RNA polymerase, a T3 RNA polymerase, a SP6 RNA polymerase and a K11 RNA polymerase.

27. (As filed) The method of claim 26, wherein said phage-encoded RNA polymerase is a mutant phage-encoded RNA polymerase that is competent to incorporate dNTPs into a template nucleic acid.

28. (As filed) The method of claim 27, wherein said phage-encoded RNA polymerase is a T7 RNA polymerase.

29. (As filed) The method of claim 28, wherein said T7 RNA polymerase contains a Y639F mutation.

30. (As filed) The method of claim 28, wherein said T7 RNA polymerase contains a S641A mutation.

31. (As filed) The method of claim 28, wherein said T7 RNA polymerase contains at least two mutations.

32. (As filed) The method of claim 7, wherein said Mn^{++} is present in a concentration of between 10 μM to 20 mM.

33. (As filed) The method of claim 32, wherein said concentration is 10 mM.

34. (As filed) The method of claim 7, wherein said target nucleic acid is single-stranded DNA.

35. (As filed) The method of claim 7, wherein the target nucleic acid is comprised of RNA.

36. (As filed) The method of claim 7, further detecting said sense transcription product, said antisense transcription product, said first amplification duplex, or said second amplification duplex,

wherein said detecting comprises hybridizing a detection oligonucleotide comprising a detectable moiety, wherein said detection oligonucleotide is complementary to a subsequence of said sense transcription product, said antisense transcription product, said first amplification duplex, or said second amplification duplex.

37. (As filed) A kit for copying a target nucleic acid comprising:

a container containing:

a first nucleotide primer comprising in the following order from 5' to 3':

a phage-encoded RNA polymerase recognition sequence,

a first spacer sequence comprising a sequence of from 12 to 20 nucleotides that consists of one nucleotide type or two different nucleotide types,

a first target complementary sequence which can bind to a segment of said target nucleic acid; and

a second primer comprising in the following order from 5' to 3':

a phage-encoded RNA polymerase recognition sequence,

a second spacer sequence comprising a sequence of from 12 to 20 nucleotides that consists of one nucleotide type or two different nucleotide types, and

a second target complementary sequence which can bind to a segment of said target nucleic acid.

38. (As filed) The kit of claim 37, wherein said phage-encoded RNA polymerase is polymerase selected from the group consisting of : a T7 RNA polymerase, a T4 RNA polymerase, a T3 RNA polymerase, a SP6 RNA polymerase and a K11 RNA polymerase.

39. (As filed) The kit of claim 38, wherein said phage-encoded RNA polymerase is a mutant phage-encoded RNA polymerase that is competent to incorporate dNTPs into a template nucleic acid.

40. (As filed) The kit of claim 38, wherein said phage-encoded RNA polymerase is a T7 RNA polymerase.

41. (As filed) The kit of claim 40, wherein said T7 RNA polymerase contains a Y639F mutation.

42. (As filed) The kit of claim 40, wherein said T7 RNA polymerase contains a S641A mutation.

43. (As filed) The kit of claim 40, wherein said T7 RNA polymerase contains at least two mutations.

44. (As filed) The kit of claim 37, further comprising a member selected from the group consisting of:

a DNA polymerase;

a Reverse Transcriptase that lacks RNAseH activity;

a phage-encoded RNA polymerase;

all four dNTPs;

those rNTPs represented in said first and second spacer sequences;

reaction buffer containing manganese in a concentration from 10 μM to 20 mM;

a positive control target nucleic acid; and

instructions for carrying out a method of copying a nucleic acid using said first primer and said second primer.